

Reproductive mode and energy costs of reproduction in the genus *Sebastes*

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The genus *Sebastes* has historically been considered to be ovoviviparous, with all energy for embryonic development coming from the yolk present at fertilization. Recent studies have shown that embryos of two species receive nutrition in addition to that supplied in the yolk. Embryonic catabolism (estimated from *in vitro* oxygen consumption) required a significant portion of the yolk energy; combined with energy content of the larva at birth, total energy was much greater than the initial egg energy. One can assess the accuracy of *in vitro* embryonic oxygen consumption by determining the excess respiration by pregnant females above that of males and immature females. In *Sebastes schlegelii*, this excess oxygen consumption is significant. The additional oxygen consumed by gestating females is greater than that predicted for oxygen consumption of the embryos early in gestation but less later in gestation, suggesting that *in vitro* estimates are approximately 80% high. We discuss the implications of these estimates to earlier calculations of viviparity. Energy taken up through ingestion and absorption of ovarian fluid in the hindgut, however, still confirms viviparity.

Introduction

Live-bearing fishes are characterized by a wide range of energetic relationships between maternal and embryonic systems. The genus *Sebastes* has historically been considered to be ovoviviparous, and that no additional nutrition is provided to the embryo during gestation (Scrimshaw 1945; Amoroso 1960). The reasons for this description have been based upon inference, however, since initial egg size is small, larvae are small at birth, and fecundity is very high (Phillips 1964; Moser et al. 1977; Boehlert et al. 1982). Recently, however,

energetic studies have demonstrated that embryos of two rockfish species receive some form of nutrition during later stages of gestation and are thus viviparous (Boehlert and Yoklavich 1984; Boehlert et al. 1986). The source of nutrition was postulated to be a nitrogenous substance derived from resorption of unfertilized ova through ingestion of ovarian fluid and subsequent uptake in the hindgut.

The work demonstrating viviparity compared indirect with direct calorimetry of embryos during development. The indirect calorimetry used *in vitro* oxygen consumption to determine catabolic needs in development. Oxygen consumption *in vitro* increases with increasing developmental stage either linearly (*S. melanops*, Boehlert and Yoklavich 1984) or exponentially (*S. caurinus*, Dygert 1986; *S. schlegell*, Boehlert et al. 1986). An important assumption of all previous work is that *in vitro* oxygen consumption is closely related to that *in vivo*. Many studies have estimated embryonic oxygen consumption of live-bearers *in vitro* (Moser 1967; Webb and Brett 1972; Berglund et al. 1986), but few have considered oxygen consumption rates *in vivo*. Boehlert et al. (1986) questioned the accuracy of *in vitro* oxygen consumption rates and suggested that they may be higher than *in vivo* rates, thus inflating estimates of catabolic energy use. In this paper we discuss the manner in which viviparity was demonstrated in *S. melanops* and *S. schlegell* and then, by considering the relative increase in oxygen consumption by gestating female *S. schlegell* as compared with spent or immature females or males (Webb and Brett 1972), provide insights to the accuracy of the *in vitro* measurements.

Materials and Methods

Methodology used in the embryonic energetics studies has been described in Boehlert and Yoklavich (1984) for *S. melanops* and Boehlert et al. (1986) for *S. schlegell* and will be only briefly described here. Developmental stages of embryos were classified according to a modification of Oppenheimer (1937) and Yamada (1963) (Kusakari unpublished). Gestation time and duration of different stages of development were determined from samples of embryos taken from females held in the laboratory. Fish were catheterized at various intervals and an analytical relationship between the stage of development and the duration of each stage was developed. From this relationship stage of development was converted to time since fertilization.

Oxygen consumption was determined for embryos at several stages of development in a Gilson differential respirometer at 10°C using standard techniques (Umbreit et al. 1972). At the end of each experiment, the embryos were counted and used for dry weight and carbon and nitrogen determinations; some embryos were preserved for determination of developmental stage. Ash-free dry weight (AFDW) was determined from groups of 35-100 embryos. Caloric content was determined from percent carbon using the nitrogen-corrected equation of Salonen et al. (1976).

For experiments on respiration rates of adult *S. schlegell*, fish from captive populations were used. Two days before beginning experiments, fish were weighed, measured, and sex determined after anesthetization in MS-222. Females were catheterized, developing embryos removed and

staged, and estimates of time since fertilization (as a function of developmental stage) were calculated following techniques of Boehlert et al. (1986).

Fish were starved at least 2 d before respiration experiments. The respirometer consisted of a cylindrical fish chamber constructed of acrylic tubing 20 cm in diameter with a volume of 13.2 liters. The respirometers were held in a 1.8 x 1.1 x 0.65 m tank which was partially covered with black plastic during experiments. Fish activity and oxygen content of the outflowing water were monitored at 5-10 min intervals. Flow-through methodology was used and oxygen concentrations determined with a polarographic oxygen electrode. Oxygen consumption rates (ml O₂/h) were determined by multiplying the change in oxygen concentration of inflowing and outflowing water by the flow rate. For each animal, a mean value was computed from two replicates. Our estimates may be considered "routine" metabolic rate (Fry 1971). Control experiments were run with empty respirometers and the drop in oxygen content was negligible.

Specimens in our experiments were divided into two groups based upon reproductive status. The first group was composed of males, immature females, and spent females and the second group females with gestating embryos at different stages of development. Respiration rates as a function of weight for the first group were fitted to a curve which could then be used as a predictive model of "normal" or non-gestating respiration rates. The estimates of this curve could then be applied to the second group; the difference between observed and predicted respiration rates was attributed to oxygen consumption by embryos within the female system plus associated costs of live-bearing.

Results and Discussion

Stage duration calculations show that early stages are passed through rapidly and that later stages, which encompass more significant morphological change, take considerably longer. The integrated relationships suggest that fertilization to birth takes 37 d for *S. melanops* (Boehlert and Yoklavich 1984) and 51.5 d for *S. schlegell*, which has a significantly larger egg (Boehlert et al. 1986). Comparisons of these estimates with data from individual females show agreement but may be somewhat variable under natural conditions. Oxygen consumption, which was determined for both species from embryos at several developmental stages, increased with time since fertilization; the relationship was nearly linear for *S. melanops* (Boehlert and Yoklavich 1984) and was exponential for *S. schlegell* (Fig. 1). For embryos in later stages of development, many hatched from the delicate chorion and limited swimming activity occurred in the respirometer flasks. This may result in inflated values of oxygen consumption for late stage embryos.

The curves, fitted to the oxygen consumption as a function of time since fertilization, were integrated to estimate the total oxygen consumed during gestation. This value can be converted to calories using an oxycaloric equivalent (Lasker 1962) to determine the total catabolic energy expenditure during gestation. A comparison of these data with the actual energy contained in the embryos (Table 1) suggests that additional energy must be used during gestation. If *Sebastes* was strictly ovoviviparous, the sum of final embryonic

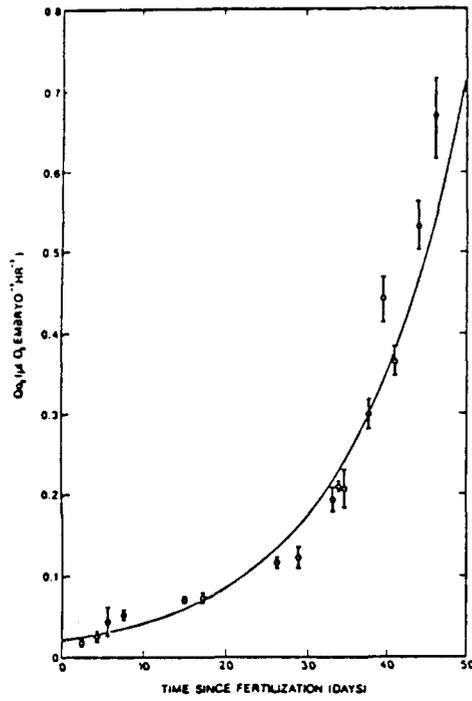


Figure 1. Oxygen consumption in embryonic Sebastes schlegell. Each point (± 2 SE) represents the mean of three or four replicates with embryos from the same female (from Boehlert et al. 1986).

Table 1. Changes in the energy content of individual embryos of Sebastes from direct estimates of caloric content compared to catabolic energy utilization estimates (from Boehlert and Yoklavich 1984; Boehlert et al. 1986).

	<u>Sebastes schlegell</u>	<u>Sebastes melanops</u>
Initial caloric content	1.59	0.43
Final caloric content	1.48	0.35
Catabolic calories	1.40	0.28

caloric content and catabolic energy utilization during gestation should equal the initial caloric content. The sum is significantly greater for both species, but more so for S. schlegell.

The relative changes in these values during gestation is graphically illustrated for S. schlegell in Figure 2. The catabolic and direct comparisons of energy utilization diverge relatively early in S. schlegell, and the results are clearly indicative of additional nutrition provided to the embryos during gestation. The catabolic energy utilization suggests that the percentages of original yolk energy remaining at birth would be 36% for S. melanops and only 12% for S. schlegell (Table 1; Fig. 2).

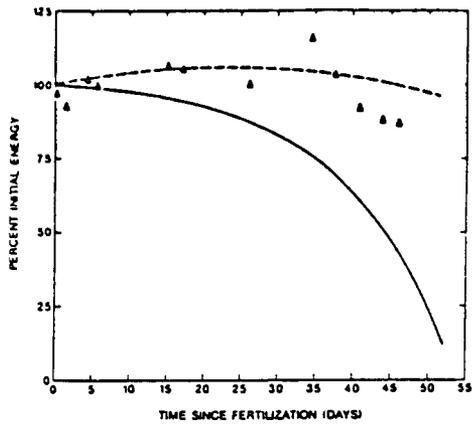


Figure 2. Summary of the energetics of development in embryonic *Sebastes schlegell*. The y-axis is the percent initial energy, where 100% represents 1.59 calories. The solid line represents expected energy remaining with time based on subtraction of cumulative catabolism. The dashed line and data points represent actual caloric content of embryos at different stages (from Boehlert et al. 1986).

As mentioned earlier, overestimating embryonic respiration could also result in overestimates of catabolic energy utilization. There are two major factors which may be possible sources of error. First, activity by later stage embryos when removed from the maternal system may increase oxygen consumption. As an example, "active" respiration rates for Pacific sardine larvae may be from 1.3 to 3.5 times those of "inactive" rates (Lasker and Theilacker 1962). Second, oxygen tension in the Gilson respirometer is initially at saturated, atmospheric levels (Umbreit et al. 1972) whereas oxygen tension inside the ovary may be low; low ambient oxygen can lower respiration rate in embryos (Carlson and Selfert 1974). It is therefore possible that the rate of development of embryos *in vivo* may be under the control of oxygen availability and that the long gestation times and stage durations may be a result. Although there is no evidence for this in *Sebastes*, Triplett (1960) noted more rapid development *in vitro* for embiotocid embryos.

Respiration measurements on adult *S. schlegell* were thus used as a check on *in vitro* measurements. Gestating fish were typically much more robust at a given length than were male, immature female, or spent females. The weight increase of gestating females over non-gestating *S. schlegell* is most marked after about 32 cm SL and the divergence between the two curves increases with increasing length, most likely due to the rapidly increasing fecundity with length. We determined oxygen consumption rates for 23 non-gestating fish, including 19 males, 2 immature females, and 2 spent females for comparison with rates for 17 pregnant females with embryos between stages 1 and 30. Respiration rates typically increased with increasing body weight for non-gestating and gestating fish (Fig. 3). Data from non-gestating fish were fitted to the curve

$$Q = 46.734 W^{0.7515} \quad n = 23 \quad r^2 = 0.524 \quad , \quad (1)$$

where Q = oxygen consumption rate (ml O_2 /h) and W = body weight (kg). At a given body weight, respiration rates were much higher for females with developing embryos. Data from these females were fitted to the curve

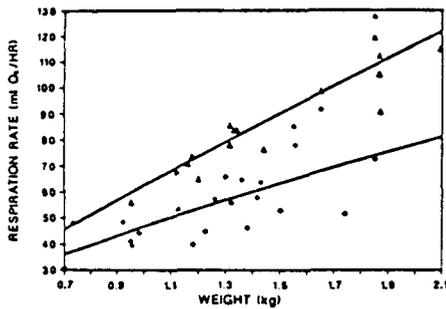


Figure 3. Oxygen consumption rates ($\text{ml O}_2/\text{h}$) as a function of fish body weight for Sebastes schlegelii. Triangles represent values from gestating females, and diamonds for males, immature females, and spent females; lines represent fitted curves (from Boehlert et al. in prep.).

$$Q = 62.383 W^{0.9014} \quad n = 17, \quad r^2 = 0.81 \quad (2)$$

These two curves (Fig. 3) are significantly different (analysis of covariance, $P < 0.01$). Differences are even more profound when comparisons are made for fish of equal length due to a much greater weight-at-length for pregnant females.

"Excess respiration" may be defined here as the amount of oxygen consumed by a gestating female in excess of that predicted for a non-gestating fish of the same length (Equation 1). This value will be the sum of embryonic respiration requirements and additional live-bearing costs, which include work associated with increased cardiac and branchial pumping and the added costs of ionic and osmotic regulation as more blood passes the gills. Calculation of this value is confounded by the weight of the female fish and the stage of development of the embryos. For this reason we standardized the measured oxygen consumption rates of gestating females (using the weight exponent in Equation 2) to a uniform weight of 1.5 kg. This value corresponds to a fish of 35.6 cm SL, with estimated post-fertilization fecundity of 126,921 embryos (Boehlert et al. 1986) and estimated total embryonic oxygen requirement (fecundity times in vitro embryonic oxygen consumption) which varied with stage of embryonic development from 3.13 to 74.86 $\text{ml O}_2 \text{ h}^{-1}$. A fish of this length in non-gestating condition would have a corresponding weight of 1.3 kg with a respiration rate of 56.92 $\text{ml O}_2 \text{ h}^{-1}$ (Equation 1). The mean excess respiration of these adjusted data is 33.39 $\text{ml O}_2 \text{ h}^{-1}$ and values show a positive relationship with total embryonic oxygen demands. Subtracting the embryonic respiration rate from the excess respiration, however, results in values which show a negative relationship with the time since fertilization (Fig. 4). This relationship suggests that the added oxygen consumption of gestating females is high early in gestation and decreases, eventually becoming negative with further embryonic development (Fig. 4). The value approaches zero at about 30 d, near the time when the mouth opens and ovarian fluid is apparently consumed (Boehlert et al. 1986). Embryos at this stage are characterized by pigmented eyes, nearly complete lens, and fully formed otoliths, rectum, and urinary bladder (Kusakari unpublished).

If we assume that activity in post-30 d embryos is a major cause of increased in vitro embryonic respiration rates, we can estimate the pattern of respiration without these data. Refitting the curve for

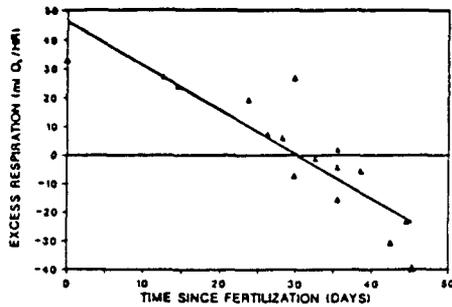


Figure 4. Elevation of respiration rate above that attributed to embryonic respiration in gestating female *Sebastes schlegell* as a function of time since fertilization. All values are standardized to a weight of 1.5 kg for the gestating female (from Boehlert et al. in prep.).

embryonic oxygen consumption (Fig. 1) with only those values less than 30 d since fertilization allows recalculation of total respiration, which would be 0.70 catabolic calories per embryo. Compared to the estimated *in vitro* catabolic utilization of 1.40 cal (Table 1), the values of *in vivo* embryonic respiration may be inflated by as much as 85%.

Respiration rates may be indirectly estimated by another method as well. Boehlert et al. (1986) suggested that the additional nutrition for embryos comes from resorption of those embryos dying early in gestation. If we assume that this is the only source of energy, then from an energy standpoint the ovaries are a closed system. This would assume that the maternal system only provides exchange of respiratory gases and metabolic waste products. This idea is supported by observed ingestion of yolk proteins by late stage embryos during a time when such materials are not present in the plasma of females (A. Takemura and K. Takano pers. commun.). The energy decrease in the ovary during gestation for the 1.5 kg female *S. schlegell* is about 95.2 kcal. This is based upon the reduction in fecundity (by embryo death) and the decreased energy content per newly hatched larva as compared to a newly fertilized egg (Table 1). Partitioned over the embryos which survive, this amounts to some 0.75 cal each, which is close to the *in vivo* catabolic estimate derived above.

From these results, it would appear that the *in vitro* estimates of catabolic energy utilization by embryos of *S. schlegell* (Boehlert et al. 1986) are high. If the value is indeed near 0.75 cal, the catabolic curve for *S. schlegell* (Fig. 2) would show about 53% of the initial energy remaining at birth, still below that estimated by direct calorimetry. Other evidence also supports viviparity. First, uptake of substances by the hindgut occurs in late stage embryos of both species studied (Boehlert and Yoklavich 1984; Boehlert et al. 1986). Further, in individual *S. schlegell* embryos, ash-free dry weight increases with time since fertilization, and significant decrease in carbon and increase in nitrogen (as a percentage of AFDW) occur during gestation (Fig. 5). The increasing AFDW and increasing percent nitrogen combine to result in a marked increase in nitrogen over the course of development, unlike most oviparous fishes, which show significant decreases in nitrogen over development (Rogers and Westin 1981).

The ovarian oxygen demand from embryonic respiration represents a significant proportion of the gestating female's excess respiration.

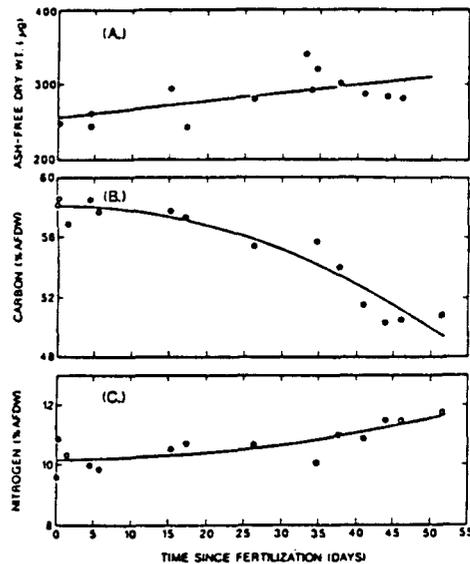


Figure 5. Changes in embryonic ash-free dry weight (AFDW) (A), carbon (B), and nitrogen (C) as a function of time since fertilization (days). Carbon and nitrogen are both expressed as percentages of ash-free dry weight (from Boehlert et al. 1986).

The other part of this excess is apparently associated with costs of live-bearing, including supply of respiratory gases and removal of waste products. That the total "excess" respiration of females during the gestation period remains fairly constant, however, suggests there is some "upper limit" for oxygen consumption. Metabolic scope may be defined as the difference between the maximum active metabolic rate supportable by aerobic metabolism and standard metabolic rate (Fry 1971); this topic has recently been reviewed by Priede (1985). Although the added weight and respiratory demands of developing embryos will contribute to a general decrease in the metabolic scope, other factors must also be considered, including general swimming activity and energy for digestion (specific dynamic action, SDA; Beamish 1974). Vahl and Davenport (1979) demonstrated an increase of 60% in the metabolic rate in *Blennius pholis* associated with a large ration and attributed this increase to apparent SDA; they suggested that in this fish and other species a single large ration may decrease the scope for activity by some 50% for several hours. Priede (1985) suggested that many fish species must time their feeding activity to keep the metabolic rate within the limits of metabolic scope. Over the extended period of gestation, *Sebastes* may need to make accommodations for the reduced metabolic scope associated with the increased respiratory load. *Sebastes* females store significant amounts of fat which are apparently depleted during the gestation period (Guillemot et al. 1985). Interannual variability in environmental factors may result in variability in the level of nutrition provided to embryos in other viviparous fishes (Trexler 1985). The effects of fish size, food availability, and physical factors thus have important implications to reproduction in the genus *Sebastes*.

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